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Comparative histological studies on the intestinal wall between the prenatal, the postnatal and the adult of the two species of Egyptian bats. Frugivorous *Rousettus aegyptiacus* and insectivorous *Taphozous nudiventris*

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KEYWORDS

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Abstract The present study was planned to find the effect of different feeding habits on the structure of the duodenum and small intestine of adult, prenatal and postnatal of both fructivorous *Rousettus aegyptiacus* and the insectivorous *Taphozous nudiventris* using the histological and the histochemical techniques. Histologically, the duodenal wall of *R. aegyptiacus* and *T. nudiventris* is composed of the typical layers: mucosa, submucosa, muscularis and serosa, we observed that the mucosa with finger like villi and very sharp apices in prenatal and adult of *R. aegyptiacus* but compact finger like villi in *T. nudiventris*. Scattered among the columnar epithelium goblet cells which less numerous in *R. aegyptiacus* than in *T. nudiventris*. Brunner's glands are less numerous also in *R. aegyptiacus* than in *T. nudiventris*. In postnatal the mucosa with pyramidal like villi in *R. aegyptiacus* and finger like villi in *T. nudiventris*. In ileum the intestinal glands are less numerous in *R. aegyptiacus* than in *T. nudiventris*. In prenatal the goblet cells are less developed in *R. aegyptiacus* and *T. nudiventris*. The intestinal glands are less developed also in *R. aegyptiacus* and *T. nudiventris* but in the postnatal the goblet cells and the intestinal gland are few in number in both *R. aegyptiacus* and *T. nudiventris*.

Introduction

The studies on bats in the world have attracted the attention of many authors. Since studies in the Egyptian bats may be few, my research focus was on the Egyptian bats. Many authors have studies on the histological and some general histochemical characteristics of gastric mucosa, small intestine, and gut associated lymphoid tissue in many different species of bats (Schultz, 1965; Rouck and Glass, 1970; Forman, 1971, 1972, 1973, 1974). Brunner's gland formed from acini and contained (PAS) positive droplets Cochrane et al. (1954).

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Forman (1974) noticed that frugivorous bats have more Payer's patches than nectarivorous, carnivorous or insectivorous ones, and that they can occur anywhere along the tract.

Physiological studies made by Keegan (1975) indicated that long intestines would not hamper the flight of frugivorous bats, since food transmit and absorption time are quite rapid. The disposition of Kerckring's folds, absent in the upper region of the duodenum and in the distal portion as it approaches the colon, is similar to that generally found in mammals.

Madkour (1977) and Madkour et al. (1982) made a comparative study of certain features of the alimentary canal and deposition of viscera in different bats from Egypt, they stated that the different parts of the alimentary tract showed a marked difference in size, shape, and length. The longest intestinal canal is found in *Rousettus*, *Nycteris*, *Rhinolophus*, and *Asellia tridens* forming a definite group as regards the length of this canal.

The Light and the Scanning electron microscopy have been used to distinguish the different portions of bat intestines (Okon, 1977; Ishikawa et al., 1985; Tedman and Hall, 1985; Makanya and Maina, 1994; Makanya, 1997; Makanya et al. 2001).

External morphology does not allow a clear distinction between the small and the large intestines in all bat species, Tedman and Hall (1985) stated that the intestine of *Pteropus alecto* and *P. poliocephalus* is a continuous tube without separation between the small and large intestines.

The gastrointestinal secretions in vertebrates contain number of muco substances that can differ according to cell type, anatomical region, functional status, pathological condition, age, sex and species (Sheahan and Jervis, 1976; Filipe, 1979; Sato and Spicer, 1980; Allen, 1981; Suganuma et al., 1981; Pedini et al., 2001; Liquori et al., 2002; Choi et al., 2003; Schumacher et al., 2004).

The intestine of *Rhinolophus ferrumequinum* is divided into three main areas: duodenum, jejuno-ileum and rectum, while the intestine of the cogeneric *Rhinolophus hildebrandti* from Africa is similar to that of *R. ferrumequinum* in having a long small intestine and a short rectum, but differs in that it lacks a distinct duodenum (Makanya and Maina, 1994).

The ileum is the final section of the small intestine, it is about 2–4 m long in man, follows the duodenum and jejunum, and is separated from the ceacum by the ileoceacal valve (Coico, 2003). The mucosa of the pangolin was noticed to be characterized by horizontal folds called plicae circulares, in contrast to abnormal finger-like projection (villi) found projecting into the lumen in rats and bats. The plicae circulares with villi increases the surface area. Further examination revealed that the muscularis externa is thicker in pangolin with rat and bat (Ofusori et al., 2008).

In *C. perspicillata*, *P. hastatus*, and *G. soricina* Peyer's patches were found in the distal portion near the large intestine, whereas in *D. rotundus* and *S. lilium* aggregations of lymphoid nodulous tissue were distributed along the tube. In *Sturnira lilium*, *Phyllostomus hastatus*, *Carollia perspicillata*, *Glossophaga soricina* and *Desmodus rotundus*, Paneth cells at the base of the crypts of Lieberkühn (Gadelha et al., 2008).

Materials and methods

Bats

The specimens of *Rousettus aegyptiacus* (Megachiroptera) and *Taphozous nudiventris* (Microchiroptera) used in this study were procured alive from Abu-Rawash, Giza Governorate through the years 2010 and 2011.

Measurements

The mean of 20 measured adult specimens in millimetre was as follows:

R. aegyptiacus: total length: 93 (88.5–95), tail: 40.5 (39.2–41.5), fore-arm: 80 (79.4–82.5), hind foot: 35.5 (34.2–36), ear: 25 (24.3–26.5).

T. nudiventris: total length: 73 (69.3–74.5), tail: 24.5 (21.1–25.6), fore-arm: 62 (60.4–63.8), hind foot: 25.5 (25.0–26.1), ear: 18 (17.5–18.5) and targus: 6 (5.8–6.3).

Collected the animals

The specimens of *Rousettus aegyptiacus* and *T. nudiventris* were collected and classified as follows:

(a) Adult group.
(b) Pre-natal group; (embryos).
(c) Post-natal group; (lactating bats) (Fig. 1).

Histological examination

Organs of adult, pre-natal, and post-natal bats (frugivores and insectivores) were collected. Parts of the duodenum, ileum, tongue, pituitary, and thyroid glands were immediately fixed in Bouin's fluid and 10% neutral buffered formalin for 24 h. The tissue samples were dehydrated in ascending grades of ethyl alcohol, cleared by xylene and embedded in paraffin. Sections of 5 μm thickness were mounted and stained with Haematoxylin and Eosin method (Bancroft and Stevens, 1990). All preparations were microscopically examined for the histological examination.

Histochemical examination

Periodic Acid Schiff procedure (PAS)

Sections were treated by Periodic Acid Schiff procedure (PAS). This comprised placing the sections in 0.5% periodic acid for 5–10 min, washing for 5 min in running tap water, then rinsed in distilled water and treated with Schiff's reagent for 20–35 min. Sections were passed through 3 changes of freshly prepared M/20 sodium acid sulphite solution, 2 min in each. They were washed in tap water for 5 min, and then rinsed in distilled water. Dehydration was carried out in ascending series of ethyl alcohols, cleared in xylene and mounted in canada balsam.

The PAS reaction is based on the oxidation of the glycol linkages in polysaccharides by periodic acid, thus producing aldehydes. These librated aldehydes react with the leuco fuchsin of Schiff's reagent producing a compound of a magenta colour. The PAS reaction was used to: demonstrate the mucous secretion of the goblet cell and the duodenal gland of both the duodenum and ileum.

Azan stain

Sections were treated by xylene for 20 min to remove the wax, hydration was carried out in descending series of ethyl alcohols, then put in aniline alcohol for 45 min, washed in acid alcohol for 1 min, put in azocarmine for an hour at 56 °C, then washed in acid alcohol and distilled water, put in phosphmolybdate for 2–3 h, washed in distilled water, put in aniline blue for an hour, washed in distilled water, put in phosphmolybedate, then in acid alcohol. Dehydration was carried out in ascending series of ethyl alcohol, cleared in xylene and mounted in canada balsam (Gretchen, 1974).

Result

Macroscopic study

In all studied bats (adult, prenatal and postnatal *T. nudiventris* and *R. aegyptiacus*) it was shown that the intestine displaced to the right of the abdominal cavity, presenting itself extremely curved (Plate 1, Fig. 1f). In *R. aegyptiacus* this curvature is more noticeable than *T. nudiventris*, and the largest part of the intestine is folded back upon itself in a compact series of numerous winding folds.

On analysing the intestine macroscopically we could not distinguish the delimitation between small and large intestines, in spite of the presence of Peyer's patches. These can be small and difficult to visualize. The duodenum can be identified by its diameter, which is bigger than the rest of the gastrointestinal tract.

The intestinal length varies greatly among the bats studied (adult, prenatal and postnatal *T. nudiventris* & *R. aegyptiacus*): *R. aegyptiacus* possessing a very long intestine and *T. nudiventris* having short intestine, the intestine length was related to body length.

Microscopic study

Histologically, the small intestinal wall of the studied bats is composed of the typical layers: mucosa, submucosa, muscularis and serosa. The mucosa characterized with a normal finger-like projection (villi) found projecting into the lumen and covered by a simple columnar epithelium constituted of absorptive cells, characterized for possessing microvilli. Scattered among the absorptive cells, the goblet cells can be found. Among the villi we found simple tubular glands, the crypts of Lieberkuhn. These glands are separated by the conjunctive tissue of the lamina propria and their extremities reach the muscularis mucosae. In the lamina propria of the villi we could see the presence of central lymphatic vessel. The Goblet cells were also found in the crypts and in the villi. The submucosa proved to be formed by slack conjunctive tissue possessing blood and lymphatic vessels.

The outer muscular layer is composed of two layers, one circular and other longitudinal. Finally, we found the serosa which is the thin, outermost layer.

Adult duodenum

Light microscopic observations

In *R. aegyptiacus* a duodenum mucosa with a finger like villi (Plate 2, Fig. 2a and c) and a compact finger like villi in *T. nudiventris* covered by a simple columnar epithelium. Scattered among the columnar epithelium the goblet cells are less numerous in *R. aegyptiacus* than in *T. nudiventris*, Brunner's glands are less numerous in *R. aegyptiacus* than in *T. nudiventris* (Plate 2 Fig. 2b and c).

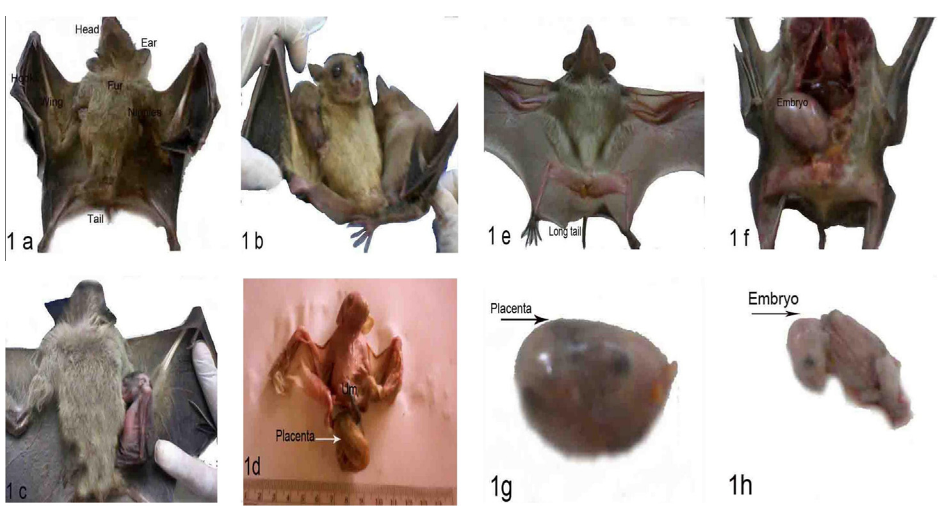

Plate 1 Photographs show external feature of studied bats. (a) External feature of adult fructivorous bat *R. aegyptiacus*. (b and c) External feature of postnatal (lactating) fructivorous bat *R. aegyptiacus*. (d) External feature of prenatal fructivorous bat *R. aegyptiacus*. (e) External feature of adult insectivorous bat *T. nudiventris*. (f) External feature of adult insectivorous bat *T. nudiventris* with embryo. (g) Prenatal bat of *T. nudiventris* inside its embryonic membranes (placenta). (h) External feature of postnatal insectivorous bat *T. nudiventris*. (i) External feature of adult tongue fructivorous bat *R. aegyptiacus*. (j) External feature of adult tongue insectivorous bat *T. nudiventris*.

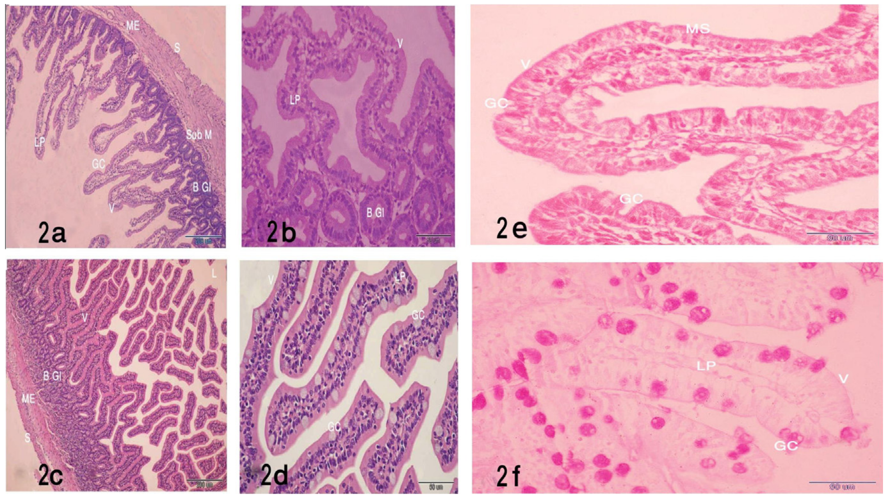

Plate 2 Photomicrographs of adult duodenum sections of *R. aegyptiacus* and *T. nudiventris*. (a and b) Transverse sections of *R. aegyptiacus* duodenum showing the muscularis layer (ME), submucosa (SU.M), Brunner's gland (BG), a finger like villi (V) and Goblet cells (G.C) H&E. (c and d) Transverse sections of *T. nudiventris* duodenum showing the muscularis layer (ME), submucosa (SU.M), Brunner's gland (BG), a compact finger like villi (V) and Goblet cells (G.C) H&E. (e) Transverse sections of *R. aegyptiacus* duodenum showing the goblet cells and the lamina propria of the villi giving strong reaction with PAS reaction. (f) Transverse sections of *T. nudiventris* duodenum showing the Goblet cells (G.C) only give strong reaction with PAS reaction.

Histochemical investigations

By using PAS stain we observe that the brush border of the columnar cells and the goblet cells gives moderate reaction in *R. aegyptiacus* (Plate 2, Fig. 2e) but in *T. nudiventris* only the goblet cells give strong PAS reaction (Plate 2, Fig. 2f).

Prenatal duodenum

Light microscopic observations

In prenatal *R. aegyptiacus* a duodenum mucosa with villi with very sharp apices and a compact villi in prenatal *T. nudiventris* covered by a simple columnar epithelium. Scattered among the columnar epithelium the goblet cells are less numerous in *R. aegyptiacus* than in *T. nudiventris*, Brunner's glands are less numerous also in *R. aegyptiacus* than in *T. nudiventris*, and the cytoplasmic contents of Brunner's glands in *T. nudiventris* are different from those in *R. aegyptiacus* (Plate 3, Fig. 3a–d).

Histochemical investigations

By using PAS stain we observe that the brush border of the columnar cells and the goblet cells gives moderate reaction but Brunner's glands give weak reaction in *R. aegyptiacus* (Plate 3, Fig. 3e), but in *T. nudiventris* the goblet cells and the Brunner's glands give strong reaction (Plate 3, Fig. 3f).

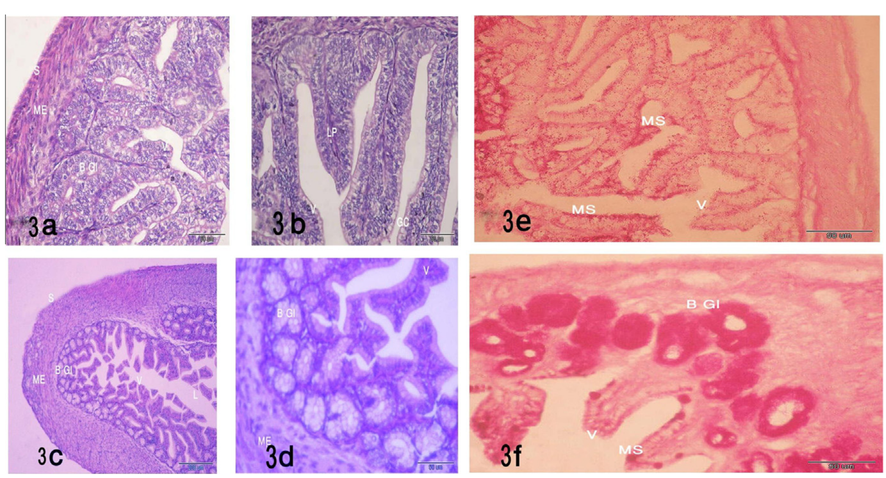

Plate 3 Photomicrographs of prenatal duodenum sections of *R. aegyptiacus* and *T. nudiventris*. (a and b) Transverse sections of *R. aegyptiacus* duodenum showing the muscularis layer (ME), submucosa (SU.M), Brunner's gland (BG), villi with very sharp apices (V) and Goblet cells (G.C) H&E. (c and d) Transverse sections of *T. nudiventris* duodenum showing the muscularis layer (ME), submucosa (SU.M), Brunner's gland (BG), a compact villi (V) and Goblet cells (G.C) H&E. (e) Transverse section of *R. aegyptiacus* duodenum showing the mucosa of the villi giving moderate reaction with PAS stain. (f) Transverse section of *T. nudiventris* duodenum showing the Brunner's glands (BG) giving strong reaction with PAS stain.

The post natal duodenum

Light microscopic observations

In postnatal *R. aegyptiacus* a duodenum mucosa with a pyramidal villi in *R. aegyptiacus* (Plate 4, Fig. 4a and c) and a finger like villi in *T. nudiventris*. Scattered among the columnar epithelium the goblet cells are less numerous in *R. aegyptiacus* than in *T. nudiventris*. Brunner's glands are less numerous also in *R. aegyptiacus* than in *T. nudiventris* and the cytoplasmic contents of Brunner's glands in *T. nudiventris* are different from those in *R. aegyptiacus*. We observe in the post natal of *R. aegyptiacus* are enormous in number than those of prenatal of *T. nudiventris* (Plate 4, Fig. 4b and c).

Histochemical investigations

By using PAS stain we observe that the brush border of the columnar cells and the goblet cells give moderate reaction and Brunner's glands give strong reaction in *R. aegyptiacus* (Plate 4, Fig. 4e and f) while in *T. nudiventris* the goblet cells and Brunner's glands give strong reaction (Plate 4, Fig. 4g and h).

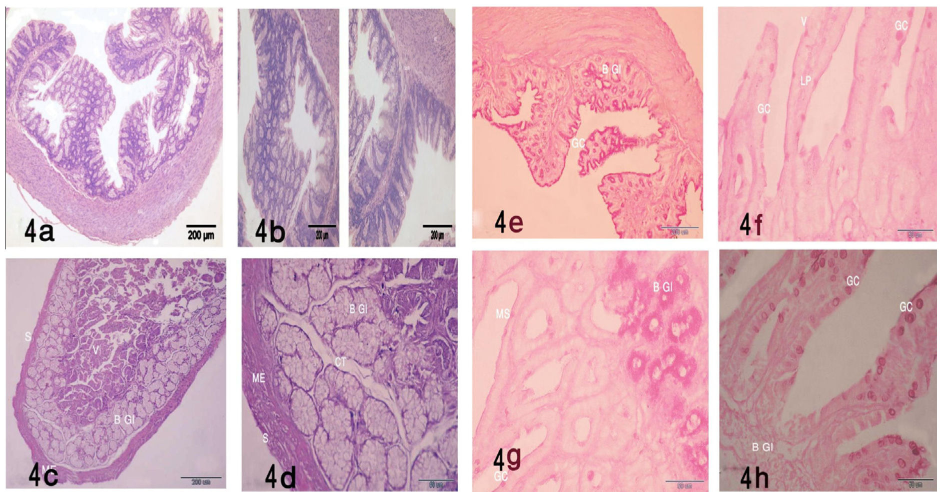

Plate 4 Photomicrographs of postnatal duodenum sections of *R. aegyptiacus* and *T. nudiventris*. (a and b) Transverse sections of *R. aegyptiacus* duodenum showing the muscularis layer (ME), submucosa (SU.M), Brunner's gland (BG), a pyramidal villi (V) and Goblet cells (G.C) H&E. (c and d) Transverse sections of *T. nudiventris* duodenum showing large numbers of Brunner's gland (BG), the muscularis layer (ME), submucosa (SU.M), Brunner's gland (BG), a finger like villi (V) and Goblet cells (G.C) H&E. (e and f) Transverse sections of *R. aegyptiacus* duodenum showing the Goblet cells and the Brunner's glands giving strong reaction with PAS stain. (g and h) Transverse sections of *T. nudiventris* duodenum showing the Brunner's glands (BG) giving strong reaction with PAS stain.

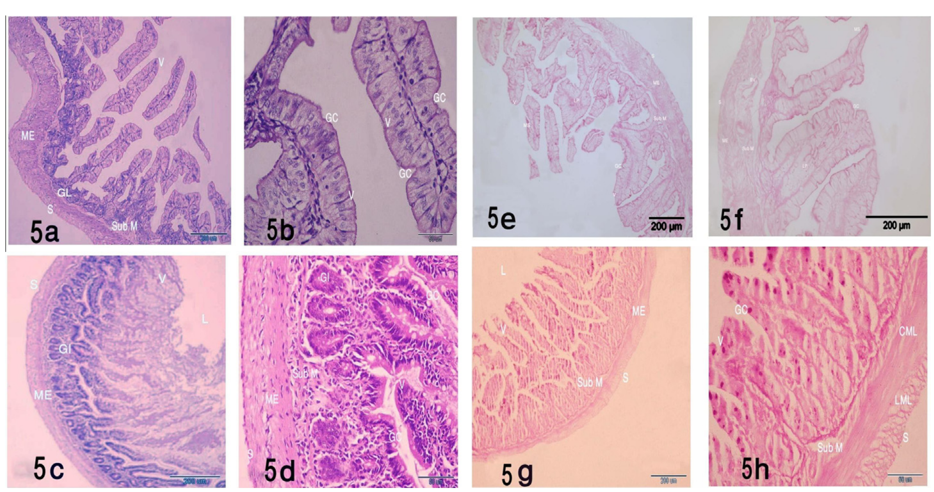

Plate 5 Photomicrographs of adult ileum sections of *R. aegyptiacus* and *T. nudiventris*. (a and b) Transverse sections of *R. aegyptiacus* ileum showing the muscularis layer (ME), submucosa (SU.M), a normal finger-like villi (V), intestinal glands (IG) and Goblet cells (G.C) H&E. (c and d) Transverse sections of *T. nudiventris* ileum showing large numbers of intestinal glands (IG), the muscularis layer (ME), submucosa (SU.M), Brunner's gland (BG), a normal finger-like villi (V) and Goblet cells (G.C) H&E. (e and f) Transverse sections of *R. aegyptiacus* ileum showing the Goblet cells and the villi giving weak reaction with (PAS) stain. (g and h) Transverse sections of *T. nudiventris* ileum showing the intestinal glands giving moderate reaction with PAS stain.

Adult ileum

Light microscopic observations

In adult *R. aegyptiacus* and *T. nudiventris* ileum the mucosa characterized with normal finger-like projection (villi) found projecting into the lumen and covered by a simple columnar epithelium constituted of absorptive cells, characterized for possessing microvilli. Scattered among the absorptive cells, the goblet cells can be found (Plate 5, Fig. 5c). The intestinal glands are less numerous in *R. aegyptiacus* than in *T. nudiventris* (Plate 5, Fig. 5a and d).

Histochemical investigations

By using PAS stain we observe that the brush border of the columnar cells (absorptive cells) and the goblet cells give moderate reaction in *R. aegyptiacus* (Plate 5, Fig. 5e and f), but in *T. nudiventris* the goblet cells and the intestinal glands give strong reaction (Plate 5, Fig. 5g and h).

The prenatal ileum

Light microscopic observations

In prenatal *R. aegyptiacus* ileum a mucosa with a finger like villi and a compact finger like villi in *T. nudiventris* (Plate 6, Fig. 6a and c). Scattered among the columnar epithelium the goblet cells are less developed in *R. aegyptiacus* and *T. nudiventris*. The intestinal glands are less developed also in *R. aegyptiacus* and *T. nudiventris* (Plate 6, Fig. 6a–d).

Histochemical investigations

By using PAS stain we observe that the brush border of the columnar cells gives weak reaction in *R. aegyptiacus* (Plate 6, Fig. 5e), but in *T. nudiventris* the brush border of the columnar cells gives stronger reaction than in *R. aegyptiacus* (Plate 6, Fig. 6f).

The post natal ileum

Light microscopic observations

In postnatal *R. aegyptiacus* ileum a mucosa with villi with sharp apices and apyramidal like villi in *T. nudiventris* (Plate 7, Fig. 7a and b). Scattered among the columnar epithelium the goblet cells are few in number in *R. aegyptiacus* and *T. nudiventris*, the intestinal glands are few in number in *R. aegyptiacus* than in *T. nudiventris* (Plate 7, Fig. 7c–f).

Histochemical investigations

By using PAS stain we observe that the brush border of the columnar cells and the goblet cells give moderate reaction in both species (Plate 7, Fig. 7g and h).

Discussion

The intestines in *R. aegyptiacus* and *T. nudiventris* are located in the same position as in the different species of Chiroptera studied by Park and Hall (1951, 1985) and Forman (1973). The intestine of adult, Prenatal and postnatal *T. nudiventris* is similar to that described by Forman (1973) displaying very little noticeable curving, while in adult, Prenatal and postnatal *R. aegyptiacus* the shape was arched as in *Sturnira lilium*, *Phyllostomus hastatus*, *Carollia perspicillata* and *Glossophaga soricina* (Schultz, 1965; Gadelha et al., 2008).

The external morphology does not allow a clear distinction between the small and large intestines in all bat species. Tedman and Hall (1985) reported that the intestine of *Pteropus alecto* and *P. poliocephalus* is a continuous tube without separation between the small and large intestines.

The micro-structural arrangement of the coat in the ileum of studied bats similar to that of mammals is a peculiar characteristic of a typical tubular organ, (Hildebrand and Goslow, 2001). The histoarchitectural modification of the mucosa of

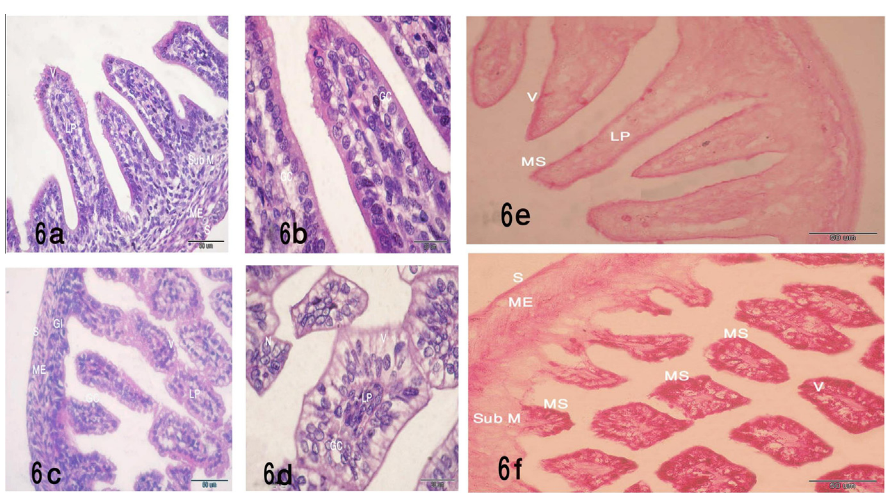

Plate 6 Photomicrographs of prenatal ileum sections of *R. aegyptiacus* and *T. nudiventris*. (a and b) Transverse sections of *R. aegyptiacus* ileum showing the muscularis layer (ME), submucosa (SU.M), a finger like villi (V), low number of intestinal glands (IG) and Goblet cells (G.C) H&E. (c and d) Transverse sections of *T. nudiventris* ileum showing large numbers of intestinal glands (IG), the muscularis layer (ME) and a compact finger like villi (V). (e) Transverse section of *R. aegyptiacus* ileum showing the mucosa of the villi giving weak reaction with PAS stain. (g) Transverse section of *T. nudiventris* ileum showing the mucosa of the villi giving moderate reaction with PAS stain.

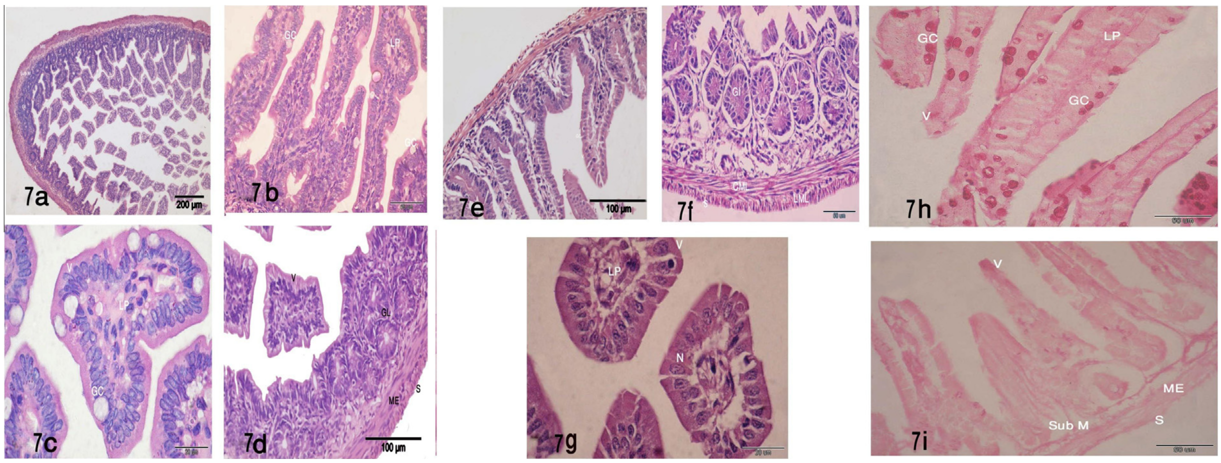

Plate 7 Photomicrographs of postnatal ileum sections of *R. aegyptiacus* and *T. nudiventris*. (a and b) Transverse sections of *R. aegyptiacus* ileum showing the muscularis layer (ME), submucosa (SU.M), A mucosa with villi with sharp apices (V), low number of intestinal glands (IG) and Goblet cells (G.C) H&E. (c and g) Transverse sections of *T. nudiventris* ileum showing large numbers of intestinal glands (IG), the muscularis layer (ME) and a pyramidal like villi (v) H&E. (h) Transverse section of *R. aegyptiacus* ileum showing the mucosa of the villi giving strong reaction with PAS stain. (i) Transverse section of *T. nudiventris* ileum showing the mucosa of the villi giving weak reaction with PAS stain.

the pangolin's ileum into plicae circulares (Ofusori et al., 2008) in contrast to studied bats is an adaptation needed to enhance the surface area and attenuate the forward flow of intra luminal contents, thus, increasing the time of contact with absorptive surfaces. This adaptation may probably be a strategy to make up for the short intestine and absent colon specially in adult, prenatal and postnatal *T. nudiventris*. These findings agreed with the works of Rubin, 1979.

Gadelha et al. (2008) stated that in *S. lilium* and *D. rotundus* a greater number of aggregates of lymphoid nodular tissue, are well distributed along the tract. In *P. hastatus* and *C. perspicillata* Peyer's patches were observed in the distal portion next to the large intestine, and the two portions of the intestine (small and large) could be distinguished. In *G. soricina* Peyer's patches could not be visualized externally, and so it was not possible to delimit the small/large intestine junction, as also observed by Ishikawa et al., 1985 in the insectivorous bat *Myotis frater*.

The absence of the caecum and of the appendix is considered characteristic of the gastrointestinal tract of chiropterans (Tedman and Hall, 1985), another feature being the absence of the ascending and transverse colons for several species of bats (Park and Hall, 1951; Ishikawa et al., 1985; Tedman and Hall, 1985; Makanya and Maina, 1994; Makanya et al., 2001), and this also applies to adult, Prenatal and postnatal *R. aegyptiacus* and *T. nudiventris*. Most bats have a smaller intestine than do terrestrial mammals of comparable size (Klite, 1965). Several comparisons of bat intestinal length measurements (Robin, 1881; Park and Hall, 1951; Forman et al., 1979; Tedman and Hall, 1985; Makanya, 1997) reveal that frugivorous species have a longer intestine, relative to body length, than species with other feeding habits.

Keegan (1975) indicated that long intestines would not hamper the flight of frugivorous bats, since food transit and absorption time is quite rapid, the gland of Brunner is easily found in the duodenum of studied bats. The villi of the small intestine mucosa presented differences, similar to the ones described by Schultz (1965) and Forman et al. (1979). The mucosa epithelium, similar to that found in other mammals, of the simple columnar type, was also observed in *M. frater* by Ishikawa et al. (1985) in *Pteropus alecto* and *P. poliocephalus* by Tedman and Hall (1985) and in *Epomophorus wahlbergi* by Makanya et al. (2001). Further, the similarities in the histo architectural configuration of the villi in studied bats are believed to be indicative of similar way of coping with their high fibre diets. The increase in length of the villi in studied bat underlines its functional implication. It appears to also play a ciliary role, which helps to complement the peristaltic movement of chyle against gravity when the animals are in their normal (up-side-down) posture.

The cell types found in the small intestine of the adult, Prenatal and postnatal *R. aegyptiacus* and *T. nudiventris* are basically the same found in mammals in general, i.e. absorptive cells which are microvilli and goblet cells which become more numerous along the tube. This was observed in other species of bats by Ishikawa et al. (1985), Tedman and Hall (1985), Makanya and Maina (1994), Makanya et al. (1995, 2001) presented less goblet cells than the *S. lilium*, *P. hastatus*, *C. perspicillata* and *G. soricinia* (Gadelha et al., 2008), a fact also observed in Pteropus alecto by Tedman and Hall, 1985 This can be associated to the type of food consumed, which is liquid and easily absorbed. The Paneth cells were observed by Schaaf (1970) in the insectivorous species group: *Artibeus jamaicensis*, *Brachyphylla nana*, *Phyllonycteris polyi* and *Monophyllus redmani*. These cells were also observed in adult prenatal and postnatal *T. nudiventris* at the base of crypts of Lieberkuhn. The muscularis mucosae and submucosa are not highly developed in the Prenatal and postnatal *R. aegyptiacus* and *T. nudiventris*, a fact also observed in *M. frater* (Ishikawa et al., 1985). Many characteristics generally found in mammals were also seen in bats, such as the presence of central lacteals in the lamina propria of the villi, and an outer muscular layer composed of a circular and a longitudinal layer. The serosa, was formed by slack conjunctive tissue covered by a mesothelium.

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